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An improved methodology of asymmetric flow field flow fractionation hyphenated with inductively coupled mass spectrometry for the determination of size distribution of gold nanoparticles in dietary supplements

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ABSTRACT

Engineered nanoparticles are available in large numbers of commercial products claiming various health benefits. Nanoparticle absorption, distribution, metabolism, excretion, and toxicity in a biological system are dependent on particle size, thus the determination of size and size distribution is essential for full characterization. Number based average size and size distribution is a major parameter for full characterization of the nanoparticle. In the case of polydispersed samples, large numbers of particles are needed to obtain accurate size distribution data. Herein, we report a rapid methodology, demonstrating improved nanoparticle recovery and excellent size resolution, for the characterization of gold nanoparticles in dietary supplements using asymmetric flow field flow fractionation coupled with visible absorption spectrometry and inductively coupled plasma mass spectrometry. A linear relationship between gold nanoparticle size and retention times was observed, and used for characterization of unknown samples. The particle size results from unknown samples were compared to results from traditional size analysis by transmission electron microscopy, and found to have less than a 5% deviation in size for unknown product over the size range from 7 to 30 nm.

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1. Introduction

The application of nanoparticles in dietary supplements is rapidly increasing due to recent innovations in the emerging field of nanotechnology [1]. One class of nanoparticle that is commonly used in dietary supplements is metallic and metal oxide nanoparticles including gold, silver, platinum, palladium, and iron oxide [2]. Absorption, distribution, and excretion of these nanomaterials in biological systems are size dependent; therefore, size based characterization is enormously significant for risk assessment [3,4]. Multiple methodologies are available for size determination of nanoparticles, including dynamic light scattering (DLS), nanoparticle tracking analysis (NTA), transmission electron microscopy (TEM), capillary electrophoresis and field flow fractionation [5]. DLS techniques provide intensity based average hydrodynamic diameter of the nanoparticles; however, its application for size determination is limited since results are biased toward the larger particles in the sample, especially when there is a broad size distribution [6,7]. Even though NTA provides a true number based size distribution, it has limited application for small nanoparticles due to its inability to visualize and track the low intensity scattered light from the smaller particles. The lower limit of detection is also material specific, which leads to varying limits of detection depending on the type of nanomaterial being analyzed, for example the lower limit for spherical metallic particles such as gold and silver is 20 nm [8]. While TEM provides a number based size distribution, its large capital equipment cost, need for an experienced operator, longer analysis and data processing times, and imaging artifacts due to sample preparation have restricted the widespread application of this technique. Furthermore, the sample size required for reasonable statistics from a TEM analysis depends on the size distribution of the particles. For example, a fairly mono dispersed sample requires the analyst to measure a moderate number of particles, while a poly-dispersed sample may require the measurement of a larger number of particles depending on the circumstances and extremely labor intensive [7]. TEM imaging is susceptible to various drying artifacts such as microfluidic coffee ring effects, Marangoni

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flow and orientation of particles based on its morphology [9]. In the automated size analysis, digital images are converted to binary images and the size is calculated by counting the number of pixels of each particle in the binary image [9]. In this case, aggregates and two adjacent particles can mistakenly be considered as a single large particle and result in erroneous measurements [9]. Therefore size-base separation and quantification techniques such as asymmetric flow field flow fractionation-inductively coupled plasma mass spectrometry (AF4-ICP-MS) and capillary electrophoresis-inductively coupled plasma mass spectroscopy are necessary for the determination of accurate size distribution [7,10–13].

AF4 has been successfully applied for the size based separation of inorganic nanoparticles, liposomes, proteins and polymers [7,14–16]. AF4 is a separation technique based on the hydrodynamic radius of an analyte. The mechanism of separation in AF4 has been explained extensively in the literature [17,18]. In brief, AF4 uses a ribbon shaped channel that is both open and narrow for separation, where the floor of the channel is lined with a porous membrane over the metal frit. The liquid flow along the narrow channel makes a parabolic flow profile with the highest velocity at the center. A cross flow is applied across the channel, forcing particles toward the porous membrane. In the separation process, diffusion brings the particles toward the center of the channel resulting in the elution of smaller particles with larger diffusion coefficients first in the fractogram. AF4 can be hyphenated with other techniques including ICP-MS, multi-angle light scattering and, UV–visible absorbance. Multiple applications of these hyphenated techniques have been demonstrated in recent literature reports [10,12,19–21].

Limited recovery due to particle accumulation on the membrane is a major hurdle for the application of AF4 as a reliable separation technique and further reduces the lifetime of the membrane [10]. To overcome this concern, we recently developed a simple particle and membrane functionalization technique specific for gold nanoparticles. Its application for the separation of commercial products is discussed in this report [10,22–24]. We demonstrated the application of AF4 hyphenated with ICP-MS and UV–visible absorption spectrometry to measure the number based size distribution of gold nanoparticles in dietary supplements. The commercial products are aqueous suspension of citrate stabilized gold nanoparticles. These results were verified with electron microscopy.

2. Experimental

2.1. Materials

Type I ultra-pure water (18 MΩ cm), obtained from a Thermo Scientific Barnstead Nanopure System (Waltham, MA) was utilized for all solution preparations. Nitric (67–69%) and hydrochloric (34–37%) acid (Optima grade) were purchased from Fisher Scientific (Houston, TX) and used for microwave-assisted digestion of samples and ICP-MS analysis. Gold (1000 mg kg^{-1}) single-element ICP-MS standard solutions were acquired from Spex CertiPrep (Metuchen, NJ) and Ultra Scientific (Kingstown, Rhode Island). Bismuth (100 mg kg^{-1}) single-element ICP-MS standard solution was purchased from Inorganic Ventures (Christiansburg, VA). Sodium chloride (ACS reagent grade), isopropanol (HPLC grade), Bis(p-sulfonatophenyl)phenylphosphine (phosphine), poly(sodium 4-styrenesulfonate) (average molecular weight: 70 kDa) were purchased from Sigma–Aldrich (Saint Louis, Mo). Gold nanoparticle reference materials with nominal diameters of 10 nm (RM8010) and 30 nm (RM8012) were purchased from the National Institute of Standards and Technology (NIST). Gold nanoparticles with nominal diameters of 5 nm, 15 nm, and 20 nm were purchased from Ted Pella (Redding, CA). Two commercially available dietary supplements that claim to contain colloidal gold nanoparticles were purchased from Internet sources. These two products (labeled as product 1 and 2) were sold as a liquid solution and were directly used for the experiment without any further purification. Millipore precut regenerated cellulose membranes with a weight cut-off of 30 kDa were purchased from Wyatt Technology (Santa Barbara, CA). Isopropyl alcohols (70%), from Cumberland Swan (Smyma, TN), and 300-mesh carbon-coated copper grids, from Electron Microscopy Sciences (Hatfield, PA), were used to prepare samples for analysis by TEM. Amicon ultra 0.5 ml centrifugal filter units with a molecular weight cut-off of 3 kDa were purchased from Millipore (Billerica, MA) and used for the removal of excess phosphine.

2.2. Functionalization and characterization of nanoparticle

Gold nanoparticle solutions were mixed with weighted amount of phosphine compound keeping phosphine concentration at 0.5 mg/mL. Gold nanoparticle standards and dietary supplements were studied on a JEOL 1400 TEM (Peabody, MA, USA) operated at 80 kV. A few drops of samples were placed directly on a 300 mesh copper grid and air dried overnight. The images were acquired by a TVIPS TemCam F416 camera and ImageJ software was used in the process of images to obtain size statistics of gold nanoparticles ($n > 1000$ for standards and $n > 3000$ for samples). Image analysis was performed with NIH ImageJ software.

2.3. Instrumental setup

Eclipse 3 AF4 systems composed of flow control unit and channel compartment (short channel with 145 mm length and 350 μm spacer, Wyatt Technology, Santa Barbara, CA) were connected to the Agilent 1200 series high performance liquid chromatography (HPLC) system, which contains a pump (G1310A), autosampler (G1329B) and diode array detector (G1315D) (Agilent Technologies, Santa Clara, CA). GASTORR AG series degasser was used to degas the buffer (FLOM Corporation, Tokyo, Japan). Millipore PVDF hydrophilic 0.1 μm membrane filter was used just after the pump. The HPLC pump was used for liquid flow into the channel compartment and the sample was introduced using autosampler. The channel flow was directed to the diode array detector using signal collection at 520 nm with 20 nm bandwidth, and 890 nm with 40 nm bandwidth as reference wavelength. An Agilent 7700X ICP-MS instrument, consisting of a quartz double pass spray chamber and glass expansion u-series sea spray nebulizer was used as the final detector. Bismuth internal standard solution (200.0 μg kg^{-1}) containing 10% HNO_3 and 10% HCl was mixed with the detector flow using a Tee junction. The voltage signal from the Agilent UV–visible detector was acquired and processed using chemstation software (B.04.03). Precut membranes were soaked overnight in 20% isopropanol, washed with Millipore water and equilibrated with carrier fluid (1 mM NaCl) in the AF4 channel for one hour. As described in our previous publication, to minimize membrane fouling 100 μL of a 5 mg/mL solution of polystyrene sufonate was injected into the system in triplicate using the same sample analysis program [10]. In all experiments, 100 μL of sample was analyzed using the methodology described in Table 1, using 1 mM NaCl as carrier fluid. Prior to the analysis, gold nanoparticle solutions including supplements were mixed with weighted amount of phosphine compound keeping phosphine concentration at 0.5 mg/mL.